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END OF SEARCH HISTORY

L3: Entry 3 of 9 File: PGPB Jul 31, 2003

PGPUB-DOCUMENT-NUMBER: 20030143682
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030143682 A1

TITLE: Antibodies and methods for generating genetically altered antibodies with high affinity

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nicolaides, Nicholas C.	Boothwyn	PA	US	
Grasso, Luigi	Bala Cynwyd	PA	US	
Sass, Philip M.	Audubon	PA	US	

US-CL-CURRENT: 435/69.1; 435/320.1, 435/326, 530/387.1, 536/23.53

CLAIMS:

We claim:

1. A method of increasing affinity of a monoclonal antibody for an antigen comprising substituting an amino acid within the variable domain of the heavy or light chain of said monoclonal antibody with a second amino acid having a non-polar side chain, whereby said affinity of said monoclonal antibody for said antigen is increased.

2. The method of claim 1 wherein said second amino acid is a proline.

3. The method of claim 2 wherein said amino acid within the variable domain of the heavy or light chain of said monoclonal antibody is an amino acid having a non-polar side chain.

4. The method of claim 3 wherein said amino acid is an alanine, or leucine.

5. The method of claim 3 wherein said amino acid is in the first framework region of the heavy chain of said monoclonal antibody.

6. The method of claim 3 wherein said amino acid is in the second framework region of the light chain of said monoclonal antibody.

7. The method of claim 2 wherein said amino acid is in position 6 of the first framework region as shown in SEQ ID NO: 18.

8. The method of claim 2 wherein said amino acid is in position 22 of the second framework region of the light chain variable domain as shown in SEQ ID NO: 21.

9. A method of increasing affinity of a monoclonal antibody for an antigen comprising substituting an amino acid within the variable domain of the heavy or light chain of said monoclonal antibody wherein said amino acid comprises a non-polar side chain, with a proline, whereby said affinity of said monoclonal antibody for said antigen is increased.

10. The method of claim 9 wherein said amino acid is an alanine, or leucine.

11. The method of claim 9 wherein said amino acid is in the first framework region of the heavy chain of said monoclonal antibody.

12. The method of claim 9 wherein said amino acid is in the second framework region of the light chain of said monoclonal antibody.

13. The method of claim 10 wherein said amino acid is in position 6 of the first framework region as shown in SEQ ID NO: 18.

14. The method of claim 10 wherein said amino acid is in position 22 of the second framework region of the light chain variable domain as shown in SEQ ID NO: 21.

15. A monoclonal antibody produced by the method of claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

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L3: Entry 5 of 9 File: PGPB May 15, 2003

DOCUMENT-IDENTIFIER: US 20030091997 A1
TITLE: Chemical inhibitors of mismatch repair

Detail Description Paragraph:
[0066] Methods for developing hypermutable cells and whole organisms have been discovered by taking advantage of the conserved mismatch repair (MMR) process of a host. Dominant negative alleles of MMR genes, when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable microbes, protozoans, insects, mammalian cells, plants or whole animals can then be utilized to develop new mutations in a gene of interest. It has been discovered that chemicals that block MMR, and thereby render cells hypermutable, is an efficient way to introduce mutations in cells and genes of interest. In addition to destabilizing the genome of cells exposed to chemicals that inhibit MMR activity may be done transiently, allowing cells to become hypermutable, and removing the chemical exposure after the desired effect (e.g., a mutation in a gene of interest) is achieved. The chemicals that inhibit MMR activity that are suitable for use in the invention include, but are not limited to, anthracene derivatives, nonhydrolyzable ATP analogs, ATPase inhibitors, antisense oligonucleotides that specifically anneal to polynucleotides encoding mismatch repair proteins, DNA polymerase inhibitors, and exonuclease inhibitors. These chemicals can enhance the rate of mutation due to inactivation of MMR yielding clones or subtypes with altered biochemical properties. Methods for identifying chemical compounds that inhibit MMR in vivo are also described herein.

CLAIMS:

1. A method for making a hypermutable cell comprising exposing a cell to an inhibitor of mismatch repair, wherein said inhibitor is an anthracene, an ATPase inhibitor, a nuclease inhibitor, a polymerase inhibitor, or an antisense oligonucleotide that specifically hybridizes to a nucleotide encoding a mismatch repair protein.

56. A method for blocking mismatch repair activity in vivo comprising exposing a cell to an anthracene compound.

L3: Entry 7 of 9 File: PGPB May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020055106
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020055106 A1

TITLE: Method for generating hypermutable organisms

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nicolaides, Nicholas C.	Boothwyn	PA	US	
Sass, Philip M.	Audubon	PA	US	
Grasso, Luigi	Philadelphia	PA	US	
Vogelstein, Bert	Baltimore	MD	US	
Kinzler, Kenneth W.	Bel Air	MD	US	

US-CL-CURRENT: 435/6; 435/325, 435/455, 435/91.1

CLAIMS:

We claim:

1. A method for generating a mutation in a gene of interest comprising the steps of: growing a hypermutable mammalian cell comprising the gene of interest and a dominant negative allele of a mismatch repair gene under control of an inducible transcriptional regulatory element; testing the cell to determine whether the gene of interest harbors a mutation; and restoring mismatch repair activity to the cell by decreasing expression of the dominant negative allele.

2. The method of claim 1 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

3. The method of claim 1 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

4. The method of claim 1 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

5. The method of claim 1 wherein the step of testing comprises analyzing the phenotype of the cell.

6. The method of claim 1 wherein the mammalian cell is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a mammalian cell, whereby the cell becomes hypermutable.

7. The method of claim 6 wherein a reporter gene interrupted with a

polymononucleotide tract which causes a reading frame-shift is introduced into the mammalian cell to permit the monitoring of hypermutability.

8. A method for generating a mutation in a mammal comprising the steps of: growing under inducing conditions one or more mammals comprising a dominant negative allele of a mismatch repair gene under control of an inducible transcriptional regulatory element; selecting one or more mammals with a new trait acquired during the step of growing; restoring genetic stability to the mammal by subjecting the mammal to non-inducing conditions.

9. The method of claim 8 wherein the new trait is identified by analyzing a nucleotide sequence.

10. The method of claim 8 wherein the new trait is identified by analyzing mRNA.

11. The method of claim 8 wherein the new trait is identified by analyzing a protein.

12. The method of claim 8 wherein the new trait is identified by analyzing a phenotype.

13. A transgenic mammal made by the method of claim 8.

14. The transgenic mammal of claim 13 wherein the mismatch repair gene is PMS2.

15. The transgenic mammal of claim 13 wherein the mismatch repair gene is human PMS2.

16. The transgenic mammal of claim 13 wherein the allele comprises a truncation mutation.

17. The transgenic mammal of claim 15 wherein the allele comprises a truncation mutation at codon 134.

18. The transgenic mammal of claim 17 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type PMS2.

19. A method for generating a mutation in a gene of interest, comprising the steps of: growing under inducing conditions mammalian cells comprising (a) a gene of interest and (b) a dominant negative allele of a mismatch repair gene under control of an inducible regulatory element; contacting the cells with a mutagen; selecting one or more cells which comprise an altered gene, an altered RNA, an altered polypeptide, or altered phenotypic trait.

20. The method of claim 19 further comprising the step of: decreasing expression of the dominant negative allele in the selected one or more cells by culturing in non-inducing conditions.

21. The method of claim 19 wherein expression of the dominant negative allele is decreased by site directed mutagenesis of the dominant negative allele.

22. A method for generating a mutation in a gene of interest comprising the steps of: treating cells comprising (a) a gene of interest and (b) a genetic defect in a mismatch repair gene with a mutagen; selecting one or more cells which comprise an altered gene, RNA, polypeptide or phenotypic trait.

23. The method of claim 22 wherein the genetic defect in the mismatch repair gene is in PMS2.

24. The method of claim 22 wherein the genetic defect in the mismatch repair gene is in PMS 1.

25. The method of claim 22 wherein the genetic defect in the mismatch repair gene is in MLH1.

26. The method of claim 22 wherein the genetic defect in the mismatch repair gene is in MSH2.

27. The method of claim 22 wherein the genetic defect in the mismatch repair gene is in GTBP/MSH6.

28. The method of claim 22 wherein the genetic defect in the mismatch repair gene is in MSH3.

29. The method of claim 22 wherein the genetic defect is a dominant-negative mutation.

30. The method of claim 23 wherein the genetic defect is a dominant-negative mutation.

31. The method of claim 22 further comprising the step of: introducing a complementing mismatch repair gene into the one or more selected cells whereby genetic stability is restored.

32. The method of claim 31 where the complementing mismatch repair gene is constitutively active in the one or more selected cells.

33. The method of claim 31 wherein the complementing mismatch repair gene is inducibly regulated.

34. The method of claim 31 wherein the complementing mismatch repair gene is in PMS2.

35. The method of claim 31 wherein the complementing mismatch repair gene is PMS1.

36. The method of claim 31 wherein the complementing mismatch repair gene is MLH 1.

37. The method of claim 31 wherein the complementing mismatch repair gene is MSH2.

38. The method of claim 31 wherein the complementing mismatch repair gene is GTBP/MSH6.

39. The method of claim 31 wherein the complementing mismatch repair gene is MSH3.

40. The method of claim 31 wherein the complementing mismatch repair gene is introduced into the one or more selected cells by cell-cell fusion with a mismatch repair proficient cell.

41. A method for measuring mismatch repair activity of a cell comprising the step of: assaying function of a gene in a cell wherein the gene comprises a polymononucleotide tract in its coding region which disrupts reading frame of the

gene downstream of the polymononucleotide tract, wherein function of the gene correlates with reduced mismatch repair activity of the cell.

42. The method of claim 41 wherein a cell with a polymononucleotide tract in the gene which does not disrupt reading frame of the gene is used as a control.

43. The method of claim 41 wherein the cell is mismatch repair defective.

44. The method of claim 43 wherein a cell is used as a control which is mismatch repair proficient.

45. A mammal comprising a dominant negative allele of a mismatch repair gene under control of an inducible transcriptional regulatory element.

46. The mammal of claim 45 wherein the mismatch repair gene is PMS2.

47. The mammal of claim 45 wherein the mismatch repair gene is human PMS2.

48. The mammal of claim 45 wherein the allele comprises a truncation mutation.

49. The mammal of claim 48 wherein the allele comprises a truncation mutation at codon 134.

50. The mammal of claim 49 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type PMS2.

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L8: Entry 1 of 2 File: USPT Dec 2, 2003

US-PAT-NO: 6656736
DOCUMENT-IDENTIFIER: US 6656736 B2

TITLE: Methods for generating hypermutable yeast

DATE-ISSUED: December 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nicolaides; Nicholas C.	Boothwyn	PA		
Sass; Philip M.	Audubon	PA		
Grasso; Luigi	Philadelphia	PA		
Vogelstein; Bert	Baltimore	MD		
Kinzler; Kenneth W.	Bel Air	MD		

US-CL-CURRENT: 435/483; 435/254.2

CLAIMS:

We claim:

1. A method for making a hypermutable yeast, comprising the step of: introducing into a yeast a polynucleotide comprising a dominant negative allele of a heterologous mismatch repair gene, wherein said heterologous mismatch repair gene is a mammalian or plant MutL homolog, whereby the cell becomes hypermutable.

2. The method of claim 1 wherein the mismatch repair gene is PMS2.

3. The method of claim 1 wherein the mismatch repair gene is plant PMS2.

4. The method of claim 1 wherein the mismatch repair gene is MLH1.

5. The method of claim 1 wherein the mismatch repair gene is MLH3.

6. The method of claim 1 wherein the mismatch repair gene is a PMSR or PMSL homolog.

7. The method of claim 2 where the allele comprises a truncation mutation.

8. The method of claim 3 where the allele comprises a truncation mutation.

9. The method of claim 2 wherein the allele comprises a truncation mutation at codon 134.

10. The method of claim 1 wherein the polynucleotide is introduced into a

yeast by mating.

11. The method of claim 2 wherein the mismatch repair gene is mammalian PMS2.

12. The method of claim 8 wherein the mismatch repair gene is plant PMS2.

13. The method of claim 1 wherein the mismatch repair gene is MLH1.

14. The method of claim 1 wherein the mismatch repair gene is MLH3.

15. The method of claim 1 wherein the mismatch repair gene is a plant MutL homolog.

16. A homogeneous composition of cultured, hypermutable yeast comprising a dominant negative allele of a mismatch repair gene, wherein said mismatch repair gene is a mammalian or plant MutL homolog.

17. The homogeneous composition of cultured, hypermutable yeast of claim 16 wherein the mismatch repair gene is a PMS2 gene or homolog.

18. The homogeneous composition of cultured, hypermutable yeast of claim 16 wherein the mismatch repair gene is a MLH1 or homolog thereof.

19. The homogeneous composition of cultured, hypermutable yeast of claim 16 wherein the mismatch repair gene is a PMSR homolog.

20. The isolated hypermutable yeast of claim 17 wherein the cells express a protein consisting of the first 133 amino acids of PMS2.

21. The isolated hypermutable yeast of claim 17 comprising a protein which consists of the first 133 amino acids of PMS2.

22. The isolated hypermutable yeast of claim 18 comprising a protein which consists of a mammalian MutL protein.

23. A method of generating a hypermutable cell comprising introducing into a cell a polynucleotide encoding PMSR2, wherein said cell becomes hypermutable.

24. The method of claim 23 wherein said cell is a yeast cell.

25. The method of claim 24 wherein said yeast cell is of a genus selected from the group consisting of Saccharomyces, Schizosaccharomyces, Pichia, Hansenula, and Kluyverornyces.

26. The method of claim 23 further comprising exposing said cell to a chemical mutagen.

27. A method for generating a mutation in a gene of interest comprising the steps of growing a cell culture comprising the gene of interest and a polynucleotide encoding a PMSR2 operably linked to expression control sequences; testing said cell to determine whether the gene of interest harbors a mutation.

28. The method of claim 27 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

29. The method of claim 27 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

30. The method of claim 27 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

31. The method of claim 27 wherein the step of testing comprises analyzing the phenotype associated with the gene of interest.

32. An isolated hypermutable cell comprising an expression vector comprising a PMSR2 coding sequence operably linked to expression control sequences.

33. A method of generating a hypermutable cell comprising introducing into a cell a polynucleotide encoding PMSR3.

34. The method of claim 33 wherein said cell is a yeast cell.

35. The method of claim 34 wherein said yeast cell is of a genus selected from the group consisting of Saccharomyces, Schizosaccharomyces, Pichia, Hansenula, and Kluyveromyces.

36. The method of claim 33 further comprising exposing said cell to a chemical mutagen.

37. A method for generating a mutation in a gene of interest comprising the steps of: growing a cell culture comprising the gene of interest and a polynucleotide encoding a PMSR3 operably linked to expression control sequences; testing said cell to determine whether the gene of interest harbors a mutation.

38. The method of claim 37 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

39. The method of claim 37 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

40. The method of claim 37 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

41. The method of claim 37 wherein the step of testing comprises analyzing the phenotype associated with the gene of interest.

42. An isolated hypermutable cell comprising an expression vector comprising a PMSR3 coding sequence operably linked to expression control sequences.

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(FILE 'HOME' ENTERED AT 10:35:25 ON 24 NOV 2004)

FILE 'MEDLINE, CANCERLIT, BIOTECHDS, CAPLUS, EMBASE, BIOSIS' ENTERED AT 10:35:44 ON 24 NOV 2004

L1	2052178	S	YEAST OR PLANT
L2	82610	S	ANTHRACENE
L3	2444	S	L2 AND L1
L4	22452	S	MISMATCH REPAIR OR HYPERMUTA?
L5	3	S	L3 AND L4
L6	2		DUP REM L5 (1 DUPLICATE REMOVED)

L6 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
AN 2003-01238 BIOTECHDS
TI Making **hypermutable** cell for agricultural, pharmaceutical or environmental applications, by exposing cell to **mismatch repair** inhibitor such as **anthracene**, ATPase inhibitor, nuclease inhibitor or polymerase inhibitor;
transgenic animal and transgenic **plant** generation by administering **anthracene** and antisense oligonucleotide
AU NICOLAIDES N C; GRASSO L; SASS P M
PA MORPHOTEK INC
PI WO 2002054856 18 Jul 2002
AI WO 2001-US934 15 Jan 2001
PRAI WO 2001-934 15 Jan 2001; WO 2001-934 15 Jan 2001
DT Patent
LA English
OS WPI: 2002-599624 [64]
AB DERWENT ABSTRACT:
NOVELTY - Making (M1) a **hypermutable** cell, comprising exposing a cell to an inhibitor of **mismatch repair** (MMR), where the inhibitor is an **anthracene**, an ATPase inhibitor, a nuclease inhibitor, a polymerase inhibitor, or an antisense oligonucleotide that specifically hybridizes to a nucleotide encoding a MMR protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) generating (M2) a mutation in a gene of interest, comprising: (a) exposing a cell or an animal comprising the gene of interest to a chemical MMR inhibitor and testing the cell or animal to determine if the gene of interest comprises a mutation; or (b) growing a **plant** comprising the gene of interest, exposing the **plant** to an inhibitor of MMR, and testing the **plant** to determine if the gene of interest comprises a mutation; (2) a **hypermutable** transgenic mammal (I) made by M2; (3) generating (M3) a MMR defective **plant** by exposing the **plant** to an inhibitor of MMR; (4) a **hypermutable plant** (II) made by M3; (5) screening (M4) for chemical inhibitor of MMR by exposing an organism to a candidate compound and screening the DNA of the organism for microsatellite instability; and (6) blocking (M5) MMR activity in vivo by exposing a cell to an **anthracene** compound.

WIDER DISCLOSURE - (1) an isolated MMR blocking chemical; and (2) a genetically altered cell line having mutation introduced through interruption of MMR.

BIOTECHNOLOGY - Preferred Method: In M1, the inhibitor is an **anthracene**. The ATPase inhibitor is a non-hydrolyzable form of ATP such as AMP-PNP. The nuclease inhibitor is an analog of N-ethylmaleimide, a heterodimeric adenine-chain-acridine compounds, or a quinilone such as Heliquinomycin. The polymerase inhibitor is an analog of aphidicolin, 1-(2'-Deoxy-2'-fluoro-beta-L-arabinofuranosyl)-5-methyluracil (L-FMAU) or 2',3'-dideoxyribonucleoside 5'-triphosphates. The antisense oligonucleotide comprises 15 consecutive nucleotides that are complementary to the coding strand of a MMR protein, where the antisense oligonucleotide specifically binds to the coding strand of the MMR protein under physiological conditions and inhibits MMR activity of the MMR protein. The antisense oligonucleotide specifically binds to a regulatory portion on the coding strand of the MMR protein. The antisense oligonucleotide is directed against the first six codons of a MMR gene message. The inhibitor of MMR is introduced into a growth medium of a eukaryotic or prokaryotic cell in vitro, or into a growth medium of a **plant**. In M2, testing comprises analyzing a polynucleotide sequence of the gene of interest, a protein encoded by the gene of interest, or the phenotype of the cell. The cell is a mammalian cell. The cell or **plant** is made MMR defective by exposing it to an MMR inhibitor. M2 further comprises removing the inhibitor of MMR after determining that the gene of interest comprises a mutation. In M2, the

animal is a mammal. M2, further comprises exposing the cell or animal to a mutagen selected from N-methyl-F'-nitro-N-nitrosoguanidine, methane sulfonate, dimethyl sulfonate, O-6-methyl benzadine, ethyl methanesulfonate, methylnitrosourea, and ethylnitrosourea. In M4, the organism is a mammal, microbe or **plant**. Screening comprises monitoring endogenous microsatellites. Screening comprises the use of reporter expression gene such as beta-glucuronidase, where the reporter expression gene comprises polynucleotide repeats within a coding region of the reporter gene. The chemical is a MMR inhibitor that induces microsatellite instability in MMR proficient cells but does not induce enhanced microsatellite instability in MMR deficient cells. Preferred **Plant**: (II) is a monocotyledon or dicotyledon.

USE - M1 is useful for making a **hypermutable** cell. M2 is useful for generating a mutation in a gene of interest. M3 is useful for generating a MMR defective **plant**. M4 is useful for screening for chemical inhibitor of MMR. M5 is useful for blocking MMR activity in vivo. (All claimed). M1 is useful for creating genetically altered host cells or organisms for agricultural, chemical manufacturing, pharmaceutical and environmental applications.

ADVANTAGE - Several advantages exist in generating genetic mutations by blocking MMR in vivo in contrast to general DNA damaging agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methylnitrosourea (MNU) and ethyl methanesulfonate (EMS). Cells with MMR deficiency have a wide range of mutations dispersed throughout their entire genome in contrast to DNA damaging agents such as MNNG, MNU and EMS and ionizing radiation. Another advantage is that mutant cells that arise from MMR deficiency are diploid in nature and do not lose large segments of chromosomes as is the case of DNA damaging agents such as EMS, MNU, and ionizing radiation. This unique feature allows for subtle changes throughout a host's genome that leads to subtle genetic changes yielding genetically stable hosts with commercially important output traits.

EXAMPLE - In order to demonstrate the global ability of a chemical inhibitor of **mismatch repair** (MMR) in host cells and organisms, human HEK293 cells (referred to as 293 cells) were treated with dimethyl **anthracene** (DMA) and measured for microsatellite instability of endogenous loci using the BAT26 diagnostic marker. Briefly, 10 to the power 5 cells were grown in control medium or 250 micro-M DMA, a concentration this was found to be non-toxic, for 14-17 days. Cells were then harvested and genomic DNA was isolated using the salting out method. Various amounts of test DNAs from HCT116 (a human colon epithelial cell line) and 293 were first used to determine the sensitivity of the microsatellite test. The BAT26 alleles were known to be heterogeneous between these two cell lines and the products migrated at different molecular weights. DNAs were diluted by limiting dilution to determine the level of sensitivity of the assay. DNAs were polymerase chain reaction (PCR) amplified using the BAT26F: 5'-TGACTACTTTTGACTTCAGCC-3' and the BAT26R: 5'-AACCATTCAACATTTTTAACCC-3' primers. PCR reactions were electrophoresed on 12 % polyacrylamide TBE gels or 4 % agarose gels and stained with ethidium bromide. These studies found that 0.1 ng of genomic DNA was the limit of detection using the conditions. To measure for microsatellite stability in 293 cells grown with or without DMA, 0.1 ng of DNA form DMA-treated or control 293 cells were amplified. Forty individual reactions were carried out for each sample to measure for minor alleles. A typical result from these studies where BAT26 alleles were amplified from DMA-treated and untreated cells and analyzed on 12 % polyacrylamide gel electrophoresis (PAGE) gels demonstrated that alleles from DMA-treated cells showed the presence of an altered allele that migrated differently from the wild type allele. No altered alleles were found in the MMR-proficient control cells as expected since microsatellite instability only occurred in MMR defective cell hosts. To confirm these data, PCRs were repeated using isolated BAT26 products. The reactions were amplified for 20 cycles using the same primers. PCR products were gel-purified and cloned into T-tailed vectors. Recombinant clones from DMA-treated and control cells were screened by the PCR again

using the BAT26 primers. Fifty bacterial colonies were analyzed for BAT26 structure by directly adding an aliquot of live bacteria to the PCR mix. PCR reactions were carried out and products were electrophoresed on 4 % agarose gels and stained with ethidium bromide. Microsatellites from DMA-treated cells had alterations that made the marker length larger or smaller than the wild type allele found in control cells. To confirm that these differences in molecular weight were due to shifts within the polynucleotide repeat, a hallmark of defective MMR, five clones from each sample were sequenced using an ABI automated sequencer with an M13-R primer located in the T-tail vector backbone. Sequence analysis revealed that the control cell clone used in the studies was homozygous for the BAT26 allele with a 26nt polyA repeat. In cells treated with DMA multiple alleles were found ranging from the wild-type with 26 polyA repeat to shorter alleles (24 polyA repeat) and larger alleles (28 polyA repeat). This data demonstrated the ability to block MMR with a chemical in a range of hosts.(114 pages)